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ABSTRACT:

PROBLEM TO BE SOLVED: To obtain an apparatus and a method in which a high quality microscope sample for diagnosis is prepared from a cell suspension with high reproducibility and without manual intervention.

SOLUTION: According to the presence or absence of continuity of an electrode 3, a level sensor 11 is turned on or the downstream side of a filter 1 is sucked by a vacuum pump 5 via a solenoid valve 10 and a constant pressure devi 1. When the end point of a filtering operation is judged, the level sensor 11 is turned off, and the solenoid valve 10 is closed as so to stop the suction operation. A cleaning-liquid supply system 6, a first fixing-liquid supply system 7 and second fixing-liquid supply system 8 are composed of tanks 61, 71, 81, of three-way selector valves 62, 72, 82 and of syringe-type pump which is driven by a motor. By this constitution, under the control of a sequencer 9, an operator charges a sample in a prescribed amount into a sample container 2. By a simple depression of a start button, an immobilized cell sample is obtained on the filter 1 after about 20 minutes.

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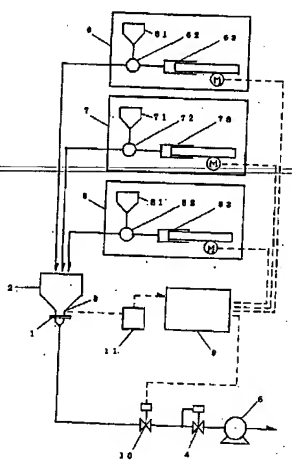
(54)【発明の名称】　自動固定標本作製装置および方法

(57)【要約】
【課題】細胞懸濁液から質の良い細胞診断用顕微鏡試料を再現性良く人手をかけないで製作する。
【解決手段】細胞懸濁液の沪過を一定の圧力の元に行うことにより、細胞の変形を防止する。沪過の終了時点を電気的に検出し、すぐに洗浄液を自動的に投入する事により、細胞の乾燥による劣化を防止する。フィルター上に捕集された細胞を洗浄を行うことによって夾雑物を除き見やすい細胞診標本を作る。一連の操作をシーケンサーにより自動化し操作条件を同一にすることにより再現性の良い標本が得られる。



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【特許請求の範囲】

【請求項１】　生体試料からなる細胞浮遊液を沪過して細胞を捕集し細胞診断用プレパラートを作成する一連の操作の前半の部分、具体的には細胞浮遊液から細胞を沪過捕集しフィルター上で細胞の固定を行うところまでの操作、を自動的に行うための装置であり、一定の吸引圧力による細胞浮遊液の吸引沪過、沪過終了を自動的に判定して吸引停止と所定量の洗浄液の自動投入、洗浄液の沪過終了を自動的に判定しての所定量の第一固定液の自動投入と第一固定化反応時間の管理、及び必要に応じての、第二固定液の自動投入と第二固定化反応時間の管理を自動的に行い、フィルター上に細胞を変性させることなく捕集し固定することを特徴とする自動固定標本作製装置。

【請求項２】　生体試料からなる細胞浮遊液を沪過して細胞を捕集し細胞診断用プレパラートを作成する一連の操作の前半の部分、具体的には細胞浮遊液から細胞を沪過捕集しフィルター上で細胞の固定を行うところまでの操作、に於いて、細胞浮遊液を沪過した後にフィルター上に残った細胞を生理的洗浄液、例えば生理食塩水、燐酸バッファー、１％ＢＳＡ含有生理食塩水等、で洗浄し付着粒子等を除去すること、及び沪過が終了した直後に生理的洗浄液を添加し、空気を吸引する事により細胞が乾燥変質する事を防止することを特徴とする細胞診断用プレパラート作成前処理方法。

【発明の詳細な説明】

【０００１】

【発明の属する技術】細胞を含む懸濁液、特に液状生体試料中から細胞を沪過捕集するに当たって、その細胞を純粋な形で、しかも変性させることなく捕集する必要がある場合に利用される。具体的には、尿中又は体くう液等の液状検体に含まれる細胞の捕集、穿刺吸引物を生理食塩水に分散させた試料からの細胞の捕集等に利用される。

【０００２】

【従来の技術】細胞懸濁液から細胞を沪過捕集する事は従来から行われていたが、殆どは手動で吸引が行われ、沪過の終了時点を肉眼で判断していた。このため吸引終了時点の判断にばらつきがあり、吸引過度で細胞がフィルターに食い込んだり、吸引不足で固定液が希釈される等の変動があり再現性が得られなかった。

【０００３】全自動固定標本作製装置としては、商品名シンプレップ（ＴｈｉｎＰｒｅｐ）が販売されている。技術内容の詳細は不明であるが、本発明との相違点は、吸引を吸引速度一定で行っているため吸引圧力一定の保証がないこと、沪過終了を吸引圧力の変化に依って判定していること、及び生理的洗浄液による洗浄を行わないことにあり、高価な装置であり処理に時間がかかるとともに多検体自動処理に不向きである。

【０００４】

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【発明が解決しようとする課題】課題の一つはきれいな顕微鏡観察のための試料が再現性良く得られること。二番目は人手の節約、特に経験を要しないで誰でも容易に良い試料が得られること。この２点が達成されれば標本作製の標準化が可能となり、細胞診断そのものの標準化が可能となる。

【０００５】

【課題を解決するための手段】きれいな顕微鏡観察のための試料を作るには一つには洗浄が有効である。フィルター上に残った夾雑物及び細胞の周囲に付いた微粒子を洗浄により除去する事によって、きれいな資料が得られ顕微鏡観察が容易になる。

【０００６】二番目には適当な吸引圧力を選定し、一定に保つことが有効である。吸引圧力が大きすぎると細胞がフィルターにめり込んで変形し、形状が変化して診断に誤差が生じる。吸引速度一定では吸引圧力が一定になる保証がなく、早くフィルターに到着した細胞と後から到着したものとの間に変形の差が生じる可能性がある。

【０００７】三番目には沪過終了の判定及び洗浄液、固定液の投入のタイミングが重要である。沪過終了の判定が遅いと空気を吸って細胞が乾燥し核の詳細な観察が不能となる。判定が早過ぎると、洗浄液の場合は問題は無いが、残留液状成分による固定液の希釈が起こり固定反応の条件が十分満足されないため、細胞が部分的に固定されるのでフィルター上に塗抹された細胞が剥離しやすくなる。また、沪過終了の判定は良くても、洗浄液、固定液の投入タイミングが遅れるとその間に細胞の乾燥がおこり良好な試料が得られない。

【０００８】以上述べたように質の良い細胞診標本を再現性良く作るには人手による操作を極力排除し、自動化することが必要であり、また自動化によって省力化がはかられるとともに、未熟練者でも、良質の試料が作成できるようになる。標本作製の標準化に当たっては多数の試料を自動的に処理できる方式が必要となる。この点にも考慮を払っておくことが必要である。

【０００９】沪過時には最適吸引圧力を一定に維持することによりフィルター上での細胞の変形を防止し、沪過終了時点を適切に判断し、洗浄液、固定液を注入する事によって細胞の乾燥による劣化を防止し、沪過終了後に洗浄液で洗浄する事によって細胞診標本の夾雑物を無くして見やすくすることができる。一連の操作を自動化することにより、これらの操作条件を再現性良く実施し、高品質の細胞診標本を再現性良く作成することができる。

【００１０】

【発明の実施の形態】尿を沪過して尿中に含まれる癌細胞を捕集するための装置を図１に示す。他の目的の装置も操作条件が異なるのみで、本質的には同一の装置が使用される。この場合は血球を透過させ、癌細胞を捕集するためフィルター１の穴径は１０ミクロン、直径１２ミ

リメートルのものを使用した。尿サンプルは５０ｍｌをサンプル容器２に投入する。本サンプル容器は下部に２本の電極３１、３２を有し、３２の下端の位置が沪過の終点判定にかかわる。この位置は実験により決定される。電極３１，３２間の導通の有無でレベルセンサー１１がＯＮ／ＯＦＦする。フィルターの下流側は電磁弁１０及び定圧装置４を経由して真空ポンプ５により吸引される。沪過の終点判定時にはレベルセンサー１１のＯＦＦで電磁弁１０が閉となり吸引を停止する。６は洗浄液供給系、７、８は第一、第二固定液供給系を示す。これらはそれぞれタンク６１，７１，８１，三方切り替え弁６１，６２，６３，モーターで駆動されるシリンジタイプのポンプから成る。９はこれらを制御するシーケンサーを示す。

【００１１】図２にサンプル容器２の詳細を示す。漏斗状のサンプル容器にアルミ箔を接着して電極３１、３２とした。３１はサンプル容器の下部まで、３２の下端は３１より少し上になるよう接着した。

【００１２】図３にシーケンサーの流れを示す。作業者はサンプル容器２に所定量のサンプルを投入しスタートボタンを押すのみで、約２０分後にはフィルター１上に固定された細胞の試料が得られる。この試料はすでに固定を終わっており、急いで次の工程（スライドガラスへの転写、染色工程、等）に進める必要はなく、ほかの作業が一段落した後に取り上げればよい。

【００１３】本装置につき各種条件を変更して実験の結果、吸引圧力は水銀柱１０ｍｍ以下、電極３２の下端の位置はサンプル容器の下端より１ｍｍが良く、洗浄液は生理食塩水、生理的燐酸バッファー（ＰＢＳ）、１％ＢＳＡを含む生理食塩水等が好結果を示し、液量としては５ｍｌで良かった。第一固定液はエタノール９５％水溶液０．５－２ｍｌ、１５分間、第二固定液は２％カーボワックスを含むイソプロピルアルコール、メタノール混合水溶液０．５ｍｌ、反応時間２分間で好結果が得られた。

【００１４】

【発明の効果】自動化により再現性良く良質の顕微鏡用試料が得られる。一定の圧力で沪過を行うことによりフィルターへの細胞の食い込み変形をなくす。沪過終了後に洗浄液による洗浄を行うことにより夾雑物のないきれいな試料が得られる。沪過終了時点を電気的に判断して吸引を停止し洗浄液を注入することにより細胞の変形と劣化を防止し質の良い試料が得られる。

【図面の簡単な説明】

【図１】本発明の装置の構成を示す。

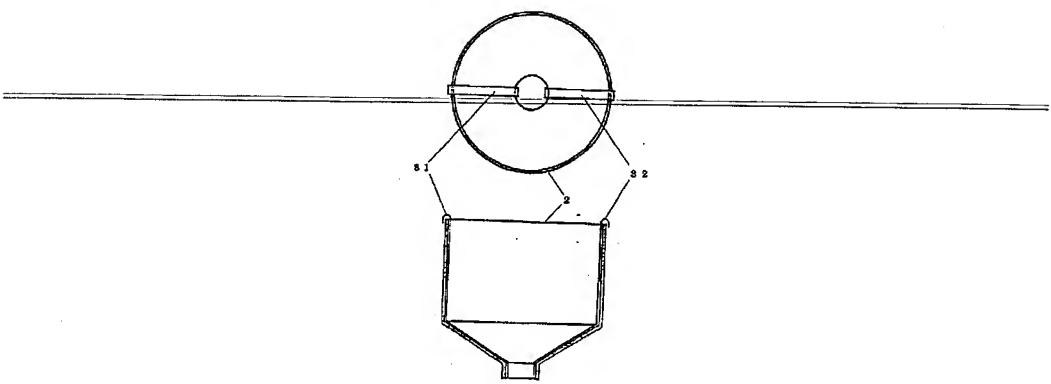
【図２】サンプル容器とそれに取り付けた電極を示す。

【図３】本装置の一連の操作の流れを示す。

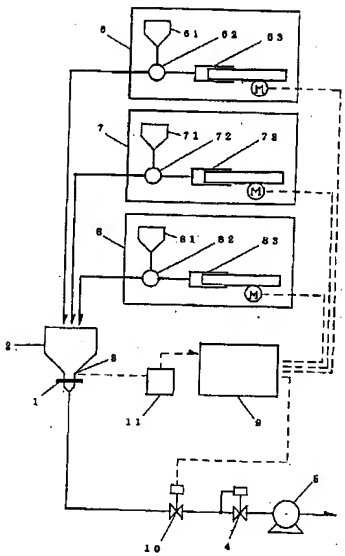
【符号の説明】

１　フィルター
２　サンプル容器
３　電極
４　定圧装置
５　真空ポンプ
６　洗浄液供給系
７　第一固定液供給系
８　第二固定液供給系
９　シーケンサー
１０　電磁弁
１１　レベルセンサー

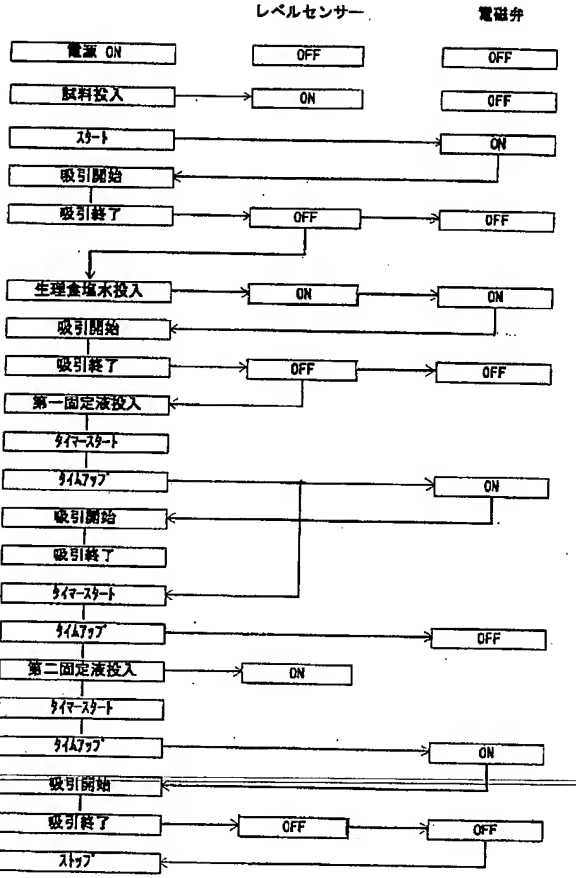
【図２】



【図1】



【図3】



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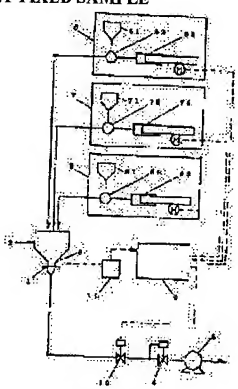
(54) APPARATUS AND METHOD FOR PREPARATION OF AUTOMATICALLY FIXED SAMPLE

(57)Abstract:
PROBLEM TO BE SOLVED: To obtain an apparatus and a method in which a high quality microscope sample for cell diagnosis is prepared from a cell suspension with high reproducibility and without manual intervention.
SOLUTION: According to the presence or absence of continuity of an electrode 3, a level sensor 11 is turned on or off. The downstream side of a filter 1 is sucked by a vacuum pump 5 via a solenoid valve 10 and a constant pressure device 4. When the end point of a filtering operation is judged, the level sensor 11 is turned off, and the solenoid valve 10 is closed as so to stop the suction operation. A cleaning-liquid supply system 6, a first fixing-liquid supply system 7 and a second fixing-liquid supply system 8 are composed of tanks 61, 71, 81, of three-way selector valves 62, 72, 82 and of a syringe-type pump which is driven by a motor. By this constitution, under the control of a sequencer 9, an operator charges a sample in a prescribed amount into a sample container 2. By a simple depression of a start button, an immobilized cell sample is obtained on the filter 1 after about 20 minutes.



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DETAILED DESCRIPTION

[Detailed Description of the Invention]

[0001]

[The technique in which invention belongs] In carrying out filtration uptake of the cell out of a liquefied biological material especially, it is used when uptake needs to be carried out without being a pure form and moreover denaturing the cell, the suspension containing a cell, and. Specifically, it is used for the uptake of the cell contained in the inside of urine, or fluid specimens, such as ********, the uptake of the cell from a sample which made the physiological saline distribute a puncture suction object.

[0002]

[Description of the Prior Art] Although carrying out filtration uptake of the cell from cell suspension was performed from the former, suction was performed manually and most had judged the termination time of filtration with the naked eye. For this reason, dispersion was in the decision at the suction termination time, a cell did not eat into a filter by suction excess, and there is fluctuation of suction being insufficient and fixing fluid being diluted, and repeatability was not acquired.

[0003] Trade name SHIMPUREPPU (ThinPrep) is sold as full automatic fixed sample production equipment. although the detail of technical contents is unknown -- the difference with this invention -- suction -- a suction rate -- since it is carrying out by being fixed -- suction pressure -- it is in that there is no fixed guarantee, having judged filtration termination therefore to change of suction pressure, and not performing washing by the physiological penetrant remover, and is expensive equipment, and while processing takes time amount, it is unsuitable for multi-specimen automatic processing.

[0004]

[Problem(s) to be Solved by the Invention] The sample for the microscope observation with one [beautiful] of a technical problem should be obtained with sufficient repeatability. A good sample should be easily obtained anyone without the second requiring saving of a help, especially experience. If these two points are attained, a standardization of sample production will be attained and a standardization of the cell diagnosis [itself] will be attained.

[0005]

[Means for Solving the Problem] Washing is effective in making the sample for beautiful microscope observation to one. By removing the particle attached to the perimeter of the impurity which remained on the filter, and a cell by washing, beautiful data are obtained and microscope observation becomes easy.

[0006] It is effective in the second to select suitable suction pressure and to keep it

constant. If suction pressure is too large, a cell will cave in and deform into a filter, a configuration changes, and an error arises in a diagnosis. suction rate regularity -- if -- there is no guarantee to which suction pressure becomes fixed, and the difference of deformation between the cell which reached the filter early, and the thing which arrived later may arise.

[0007] The timing of an injection of a judgment and penetrant remover of filtration termination, and fixing fluid is important for the third. If the judgment of filtration termination is slow, air will be inhaled, a cell will dry and nuclear detailed observation will serve as impossible. When a judgment is too early, and it is a penetrant remover, it is satisfactory, but since dilution of the fixing fluid by the residual liquid-like component takes place, the conditions of a fixed reaction are not satisfied enough, and a cell is fixed partially, the cell by which the smear was carried out on the filter becomes easy to exfoliate. Moreover, even if the judgment of filtration termination is good, if the injection timing of a penetrant remover and fixing fluid is overdue, desiccation of a cell will start between them and a good sample will not be obtained.

[0008] While it is required for making the high quality cytologic specimen with sufficient repeatability to eliminate actuation by the help as much as possible, and to automate as stated above, and laborsaving is achieved by automation, a good sample can be created also by the unskilled man. The method which can process many samples automatically in a standardization of sample production is needed. It is required also for this point to pay consideration.

[0009] By maintaining the optimal suction pressure uniformly at the time of filtration, by pouring in a penetrant remover and fixing fluid, deformation of the cell on a filter can be prevented and a filtration termination time can be judged appropriately, and degradation by desiccation of a cell is prevented, and by washing by the penetrant remover after filtration termination, the impurity of the cytologic specimen can be lost and it can be made legible. By automating a series of actuation, these operating conditions can be carried out with sufficient repeatability, and the cytologic specimen of high quality can be created with sufficient repeatability.

[0010]

[Embodiment of the Invention] The equipment for carrying out uptake of the cancer cell which filters urine and is contained in urine is shown in drawing 1 . Only by operating conditions differing also in the equipment of other purposes, the same equipment is essentially used. In this case, the corpuscle was made to penetrate, and in order to carry out uptake of the cancer cell, the bore diameter of a filter 1 used 10 microns and a thing with a diameter of 12 millimeters. A urine sample supplies 50ml to the sample container 2. This sample container has two electrodes 31 and 32 in the lower part, and the location of the lower limit of 32 is concerned with the terminal point judging of filtration. This location is determined by experiment. A level sensor 11 carries out ON/OFF by the existence of the flow between an electrode 31 and 32. The downstream of a filter is attracted by the vacuum pump 5 via a solenoid valve 10 and barostat 4. At the time of the terminal point judging of filtration, a solenoid valve 10 serves as close in OFF of a level sensor 11, and suction is stopped. 6 shows seven and a penetrant remover supply system and 8 show the second fixing fluid supply system for a start. These consist of the pump of the syringe type driven by tanks 61, 71, and 81, the Mikata change valves 61, 62, and 63, and the motor, respectively. 9 shows the sequencer which controls these.

[0011] The detail of the sample container 2 is shown in drawing 2 . Aluminum foil was pasted up on the funnel-like sample container, and it considered as electrodes 31 and 32. To the lower part of a sample container, 31 pasted up the lower limit of 32 so that it might turn from 31 up for a while.
[0012] The flow of a sequencer is shown in drawing 3 . An operator supplies the sample of the specified quantity to the sample container 2, it is only pushing a start button and the sample of the cell fixed on the filter 1 is obtained after about 20 minutes. What is necessary is for this sample to already have finished immobilization and not to advance it to the following processes (the imprint to slide glass, dyeing process, etc.) in a hurry, and just to take it up, after other activities are settled temporarily.
[0013] Various conditions were changed per this equipment, as a result of the experiment, 1mm of suction pressure is [the location of the lower limit of 10mm or less of mercury gages, and an electrode 32] better than the lower limit of a sample container, and the penetrant remover's was [the physiological saline, the physiological phosphoric acid buffer (PBS), the physiological saline that contains BSA 1% showed the good result, and] good at 5ml as volume. The good result was obtained in [isopropyl alcohol and 0.5ml of methanol mixed water solutions, and reaction-time] 2 minutes. [with which the second fixing fluid contains Carbowax 2% for a 0.5 to 2 ml ethanol 95% water solution, and 15 minutes in the first fixing fluid]
[0014]
[Effect of the Invention] The good sample for microscopes with sufficient repeatability is obtained by automation. Interlocking deformation of the cell to a filter is abolished by filtering by the fixed pressure. A beautiful sample without impurity is obtained by performing washing by the penetrant remover after filtration termination. By judging a filtration termination time electrically, stopping suction, and pouring in a penetrant remover, deformation and degradation of a cell are prevented and a high quality sample is obtained.

[Translation done.]

NOTICES *

DESCRIPTION OF DRAWINGS

[Brief Description of the Drawings]
[Drawing 1] The configuration of the equipment of this invention is shown.
[Drawing 2] A sample container and the electrode attached in it are shown.

[Drawing 3] The flow of a series of actuation of this equipment is shown.
[Description of Notations]
1 Filter
2 Sample Container
3 Electrode
4 Barostat
5 Vacuum Pump
6 Penetrant Remover Supply System
7 First Fixing Fluid Supply System
8 Second Fixing Fluid Supply System
9 Sequencer
10 Solenoid Valve
11 Level Sensor

[Translation done.]
* NOTICES *

1.This document has been translated by computer. So the translation may not reflect the original precisely.
2.**** shows the word which can not be translated.
3.In the drawings, any words are not translated.

CLAIMS

[Claim(s)]
[Claim 1] The part in the first half of a series of actuation which filters the cell suspension which consists of a biological material, carries out uptake of the cell, and creates the prepared slide for a cell diagnosis, The actuation by the place which specifically carries out filtration uptake of the cell from cell suspension, and fixes a cell on a filter, The suction filtration of cell suspension are equipment for carrying out automatically and according to fixed suction pressure, Filtration termination is judged automatically. The automatic injection of the penetrant remover of a suction halt and the specified quantity, An automatic injection of the first fixing fluid of the specified quantity which judges filtration termination of a penetrant remover automatically, and management of the first fixed reaction time, And automatic fixed sample production equipment characterized by carrying out uptake and fixing, without performing automatically automatic injection of the second fixing fluid if needed and management of the second fixed reaction time, and denaturing a cell on a filter.
[Claim 2] The part in the first half of a series of actuation which filters the cell suspension which consists of a biological material, carries out uptake of the cell, and creates the prepared slide for a cell diagnosis, The actuation by the place which specifically carries out filtration uptake of the cell from cell suspension, and fixes a cell

on a filter, it being alike and setting, a physiological penetrant remover, for example, a physiological saline, a phosphoric acid buffer, 1%BSA content physiological saline, etc. coming out, washing the cell which remained on the filter after filtering cell suspension, and removing an adhesion particle etc. -- And the prepared slide creation pretreatment approach for a cell diagnosis characterized by preventing that a cell carries out desiccation deterioration by adding a physiological penetrant remover immediately after completing filtration, and attracting air.

[Translation done.]